RESEARCH NOTE

DETECTION OF Chlamydophila felis ANTIBODIES IN Felis catus and Panthera tigris AT A WILDLIFE FACILITY USING ELISA

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ABSTRACT

Thirty domestic short haired cats (*Felis catus*) and nine tigers (*Panthera tigris*) of both sexes and various ages, living within the vicinity of a wildlife facility were used in the study. These animals were apparently healthy at the time of the study. Only the tigers were vaccinated against Rabies, Feline Panleukopenia, Calicivirus and Herpesvirus and dewormed with ivermectin. The blood sera of these animals were tested for *Chlamydophila felis* antibodies using an ELISA test kit. Six of the 39 (15%) animals tested had serologic evidence of exposure to *Cp. felis*. Three of the 30 (10%) domestic short haired cats and three out of the nine tigers (33%) tested positive. Adults (83%) were found to be more prone to exposure than juveniles (17%). Both male and female have equal predisposition to exposure to *Cp. felis*. The study shows that adult felids were more prone to the infection than young felids, regardless of sex.

Keywords: cat, Chlamydophila felis, ELISA, tiger

INTRODUCTION

Chlamydophila felis (*Cp. felis*) is an obligate intracellular bacterium that causes acute or chronic conjunctivitis in cats. The organism was previously known as *Chlamydia psittaci var. felis* until Everett *et al.* (1999) published a revision of the taxonomy of the family Chlamydiaceae. *Cp. felis* is a Gram-negative rod-shaped coccoid bacterium (Gruffydd-Jones *et al.*, 2009) that replicates in the epithelial mucosa of the upper and lower respiratory tract (Acha and Szyfres, 1994). Transmission requires close contact between cats, with ocular and nasal secretions being the most important bodily fluid for infection (Acha and Szyfres, 1994; Gruffydd-Jones *et al.*, 2009).

The pathogenesis of feline chlamydophilosis remains largely unknown (Sykes, 2005); however, the primary target of *Cp. felis* is the conjunctiva (Gruffydd-Jones *et al.*, 2009). Natural transmission presumably occurs by close contact with other infected cats and aerosolized secretions, via fomites (Sykes, 2005). The incubation period is generally 2-5 days (Gruffydd-Jones *et al.*, 2009). The infection shows chronic watery ocular or nasal discharge that may last for weeks and months, chronic bronchitis and pneumonia (Norsworthy, 1993), intense conjunctivitis with extreme hyperemia of the nictitating membrane, blepharospasm and ocular discomfort (Radolakis and Mohamad, 2010).

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Chemosis of the conjunctiva is a characteristic feature of the infection (Gruffydd-Jones *et al.*, 2009).

Detection of *Chlamydophila felis* antibodies in cats and tigers

The prevalence of *Cp. felis* infection is highest in young cats (TerWee *et al.*, 1998) and the disease tends to be more severe in kittens (Wills and Wolf, 1993). In contrast, Gunn-Moore *et al.* (1995) reported that adult cats were more likely to be exposed to the organism. Seki *et al.* (2009) reported that there is no significant difference between sex and seroprevalence.

Cp. felis infection may be associated with human conjunctivitis (Sykes, 2005). Hartley (2001) reported the recovery of *Cp. felis* in a 39-year old HIV-positive patient with a history of chronic conjunctivitis. Apparently, the same organism was isolated from the six cats owned by the same individual. Because of the zoonotic potential, proper hygiene and sanitation, and precaution should be exercised by people handling these animals (Travnicek *et al.*, 2002). A survey of published references showed that there is no information regarding the prevalence and *Cp. felis* antibody detection in domestic and wild felids in the country; hence, this study was conducted.

MATERIALS AND METHODS

Thirty (30) domestic short haired cats (*Felis catus*) of both sexes and varying ages, from a wildlife facility in Manila, Philippines were used in the study. The diet, records and method by which the domestic cats were captured were as described by Reyes *et al.* (2013). The cats that were apparently healthy after the study were placed for adoption; otherwise, they were humanely destroyed.

Also, nine tigers (five males and four females) were used in the study. There were actually 10 tigers in the facility, however, one of the female tigers was lactating at the time of the study, hence, was excluded to prevent stress. The adult tigers were either confiscated or donated by private owners; thus, the exact source and history of exposure to other felines and other animals prior to zoo residency are unknown. These animals were dewormed twice a year with Ivermectin (Ivomec®1%; Merial Inc.) and vaccinated annually against Rabies, Feline Panleukopenia, Calici virus and Herpesvirus. Diet was composed of carabeef and chicken and water is provided *ad libitum*.

These animals were kept in a large communal enclosure, however, individual enclosure made of concrete floors and wall and steel bar doors were available for isolation and feeding. These enclosures were cleaned daily with water. There were no apparent disease outbreaks reported and all the tigers were apparently healthy during the study.

The experimental methods described herewith were approved and permitted by the University's Institutional Animal Care and Use Committee (IACUC).

Prior to blood collection, the animals were placed in individual holding cages. Chemical restraint using tiletamine hypochloride-zolazepam hypochloride (Zoletil ®, 50mg/ml, VirbacLaboratories, Carros, France) was done. The cats received chemical restraint at a dose of 5 mg/kg administered intramuscularly (IM), while the tigers were given approximately 10 ml to effect using a dart propelled by a compress dart gun.

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Approximately five (5) ml of blood were collected using 23-gauge hypodermic needle attached to a 5 ml disposable syringe from all the animals via lateral saphenous venipuncture or jugular venipuncture for the tigers and cats, respectively. The blood samples were placed in a red capped vacuum tube (Vacutainer). Each sample was labelled with the animal code, sex and age and was allowed to stand for at least 30 min.

The sera were harvested after centrifugation and were tested using commercially available ELISA test kit (Immunocomb[®] ELISA Feline *Toxoplasma* and *Chlamydophila* Antibody Test Kit, Biogal Laboratories, Kibbutz, Galed, Israel). Interpretation was made using computer software (CombScan® 2000, Biogal Laboratories, Kibbutz, Galed, Israel). The correlation of sex and age to the *Cp. felis* antibody titer were analyzed using Spearman's Test of Independence.

RESULTS AND DISCUSSION

Thirty domestic short haired cats and nine captive tigers in a wildlife facility were used in the study. The health conditions of these animals were assessed and were found to be apparently healthy during the duration of this study. None of the animals showed signs of infection of respiratory distress.

The results showed that 15% (6/39) of the animals tested had serologic confirmation of previous but undetermined point in time of exposure to *Cp. felis* (Table 1). These values are comparable to reported seroprevalence in Sweden (11.5%), Australia (14.3%), Britain (17.7%) and Italy (20%) (Sykes, 2005). Furthermore, the results of this study are lower than the report made by Tuzio *et al.* (2005) in Japan where infection is between 15-51.1% in domestic and stray cats, respectively.

Of the animals that tested positive for *Cp. felis* antibodies, 10% (3/30) were domestic short haired cats and 33% (3/9) were captive tigers. Transmission of the organism is via ocular and nasal discharge (Sykes, 2005). With the domestic short haired cats' unknown vaccination record and history of disease and infection, it is highly possible that the

Table 1. Frequency distribution of domestic short haired cats (n=30) and captive tigers (n=9) with serologic evidence of exposure to *Cp. felis* by sex.

	Domestic short haired cats				Total		
Interpretation	Male	Female	Total	Male	Female	Total	TOLAI
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Seropositive	1/15	2/15	3/30	2/5	1/4	3/9	6/39
(>1:16)	(6.67)	(13.33)	(10)	(40)	(25)	(33.33)	(15.38)
Seronegative	14/15	13/15	27/30	3/5	3/4	6/9	33/39
(≤1:16)	(93.33)	(86.67)	(90)	(60)	(75)	(66.67)	(84.61)
Total	15	15	30	5	4	9	39

traffic and contact of these animals may have infected the captive tigers. According to Oronan *et al.* (2013), civet cages situated next to each other may have contributed to the transmission and spread of the disease. Moreover, the absence of records of the tigers makes it impossible to recall previous infections and diseases, including *Cp. felis* infection that these tigers might have had.

A higher prevalence was found in tigers compared to the domestic cats. This could be due to the fact that the tigers are confined in an area and may be continuously exposed to the organism via fomites (Sykes, 2005). Table 1 also shows that there is no difference between sex and the presence of *Cp. felis* antibodies. The results in this study agree with what was reported by Seki *et al.* (2009).

Table 2 shows that exposure rate to *Cp. felis* increases with age where 83% (5/6) of the animals with evidence of exposure to *Cp. felis* were adults and 17% (1/6) was juvenile. This is in contrast with the reports made by TerWee *et al.* (1998) and agrees with Gunn-Moore *et al.* (1995). Most infections, in domestic and wild felids occur post- weaning, when these animals start to hunt or look for food (Lucas *et al.*, 1999). Reyes *et al.* (2013) reported a higher frequency in adult animals as these animals have an inclination to roam.

The results of this study confirmed the presence of *Chlamydophila felis* antibodies among the domestic short haired cats and captive tigers at a wildlife facility in Metro Manila.

	Domestic short haired cats			Captive tigers			Total
Interpretation	Juvenile	Adult	Total	Juvenile	Adult	Total	TOLAI
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Seropositive	0/3	3/27	3/30	1/3	2/6	3/9	6/39
(>1:16)	(0)	(11.11)	(10)	(33.33)	(33.33)	(33.33)	(15.38)
Seronegative	3/3	24/27	27/30	2/3	4/6	6/9	33/39
(≤1:16)	(100)	(88.89)	(90)	(66.67)	(66.67)	(66.67)	(84.61)
Total	3	27	30	3	6	9	39

Table 2. Frequency distribution of domestic short haired cats (n=30) and captive tigers (n=9) with serologic evidence of exposure to *Cp. felis* by age.

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